Electron Microscopic Studies on the Human Non-Epithelial Malignant Tumors

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by

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INTRODUCTION

The ultramicroscopic structure of cells has been greatly clarified by recent studies of various modes of intracellular virus multiplication. The presence of viruses multiplying in the cytoplasm of certain animal tumors has been demonstrated by the ultrathin section method as well as in well-recognized human viral tumors such as warts and molluscum contagiosum. On the other hand, it was noted that virus particles appear in the cells of SHOPE's rabbit papilloma only after proliferation has ceased and keratinization has started. In chicken sarcoma, it was found that virus particles develop only along the cell membrane of sarcomatous cells and not intracellularly[1)2)]. Cancer of the breast and leucemia in mice and several other malignant animal tumors also reveal this distribution of virus particles. Therefore, it can no longer be doubted that viruses are the causative agents of some malignant animal tumors.

However, virus particles have been demonstrated in only a low percentage of viral malignant animal tumors[3)4)]. For example, virus particles are very difficult to be proved by electron microscopic observation even in chicken sarcoma[3)4)], a wellknown viral malignant tumor, demonstration being successful in only 0.32% of the "Chiba strain". The same sarcoma cells revealed the virus in 36% after X ray irradiation with 200 γ, in 18% after treatment with methylcholanthrene, and in 7.6% after the administration of nitromin (Methyl•bis-(β-chloroethyl)—amine—N—oxied•hydrochloride)[5)]. These facts indicate that certain conditions or pretreatment may be required for the demonstration of virus particles in human malignant tumors by the electron microscope.

Virus-like particles were reported in the cells of human malignant tumors by DMOCHOWSKI[6)7)] BESIS[8)] and others. Recently, Z. OTA[9)] (Okayama Universty) claimed that the same entities were found in chloroleucemia cells. On the other hand, K. OTA[10)] (Tokyo Medical & Dental College) failed to show virus-like particles in his study of 14 human non-epithelial benign tumors (1 angioma, 4 fibromas, 2 leiomyomas, 3 ganglioneuromas, and 4 neurinomas) and 12 non-epithelial malignant human tumors (4 angiosarcomas, 2 fibrosarcomas, 1 leiomyosarcoma, 3 rabdomyosarcomas, 1 malignant neurilemmoma and 1 neuroblastoma).

The author looked for virus-like particles in 35 human tumors thought to be non-epithelial malignant tumors: 4 enlarged lymphnodes 5 carcinomas, 1 malignant mixed tumor, 2 benign non-epithelial tumors and a benign mixed tumor. Electron microscopic

studies of these tumors revealed intracytoplasmic virus-like particles only in the lymphnode metastasis of synovial sarcoma and in a malignant mixed parotid tumor.

MATERIAL AND METHODS

Materials were extirpated and fixed in the operating room before or as soon as possible after ligation of the main blood vessels, but in some instances as long as 20 minutes elapsed before fixation due to prolonged ligation.

Fixation was carried out for 90-120 minutes in DALTON's 2 % chrome-osmium solution[11)12)] at 3°C, followed by dehydration for 15 minutes each in 50%, 70%, 90%, and 95 % ethanol and then twice for 20 minutes each in 100 % ethanol. Materials were macerated twice for 10 minutes each in propylene oxide and two volumes of embedding material (Epon) so as to cause adequate penetration into the tissue. Polymerization was completed by allowing the embedded materials to stand for 24 hours at 37°C and for 24 hours more at 60°C.

The Epon used was of the following composition: 5 ml Epon 812, 5 ml Epon 815, 16 ml D. D. S. A. and 0.45 ml D. M. P. 30[13)~17)]. The tissue was cut with a glass-knife and sliced with a Leiz ultramicrotome to a thickness of about 0.05 μ. The ultrathin sections were layed over a mesh covered with carbon-coated collodion membrane and observed with a Hidachi HS 6 electron microscope. Some sections remained unstained and others were stained with lead-hydroxide[18)19)] or with uranyl-acetate[20)21)].

OBSERVATION

(1) Synovial Sarcoma

A 34-year-old woman was admitted to this hospital on December 9, 1961, suffering from a painful walnut-sized swelling over the right internal malleolus. The tumor was removed on January 24, 1962, and proved histologically to be synovial sarcoma. Roentgen treatmant (24 times, totaling 6,000 γ) and tespamin (N•N′•N″-Triethylen Thiophosphoramide) (14 injections of 5 mg each) were given post-operatively. Subsequently, her right inguinal lymphnodes became enlarged and were removed on July 25. The specimen described in this report was from this lymphnode metastasis.

Electron microscopic observation demonstrated that most nuclei were irregular in shape, contained electron-dense granules and had double nuclear membranes. One or 2 nucleoli, could be seen distinctly. Mitochondria of various shapes and sizes were poorly developed in the cytoplasm, which was relatively scant as compared with that in healthy cells. The development of endoplasmic reticulum was inhibited and many vacuoles were seen in the cytoplasm. It was especially noted that a number of electron-dense particles were gathered in the cytoplasm, and some of them appeared even extracellulary. These particles measured 70-80 mμ, approximately the size of virus particles previously identified in malignant tumor cells. However, the unit membrane—a characteristic of virus particles—could not be seen in these particles.

The particles stained sharply with lead-hydroxide and even more strongly with uranyl-acetate. This observation is worth mentioning, since virus particles are known to have a strong affinity for these two stains.

(2) Malignant Mixed Parotid Tumor

In 1950, a 40-year-old woman first noticed a lump, the size of a grain of rice under her ear. This non-painful tumor grew slowly for many years, and then began to grow rapidly after the autumn of 1961. It was removed on January 24, 1962.

Electron microscopic examination revealed somewhat irregular nuclei with double nuclear membranes and a few well-defined nucleoli. The cytoplasm was poorly developed, but more abundant than in the synovial sarcoma metastasis mentioned above. Normal growth of mitochondria, poorly developed endoplasmic reticulum and many intracytoplasmic vacuoles were noted. In the cytoplasm of the tumor cells there were aggregates of electron-dense particles. The particles were 25-30 mμ in diameter and the aggregates, 70-80 mμ. They showed a strong affinity for lead-hydroxide and uranyl-acetate, especially the latter.

No electron-dense particles were seen intranuclearly in either case.

DISCUSSION

OBERLING, BERNHAND and others[22)23)24)] reported that cells from cancer of the breast in C_3H mice contained two kinds of virus particles—one 50 mμ and the other 80 mμ in diameter. They stated that the smaller particles appeared in the cytoplasm of carcinomatous cells, gathered together in groups and moved towards protrusions of the cell surface where they finally formed large-sized particles. DMOCHOWSKI, Moore and others[25)26)] studied virus particles in the same animal carcinoma, but failed to show the transformation from small particles to larger ones. SUZUKI[27)] observed that ripe particles were discharged from the intracellular unripe ones towards the cell surface. He illustrated the course of this transformation clearly in "Gann" (Japanese Journal of Cancer Research).

On the other hand, AMANO et al[1)2)] claimed, on the basis of their observations of breast carcinoma in C_3H and in SL mice that the superficial virus particles originated from the top of microvilli of the cytoplasm located along the duct lumen. They stated also that a transparent axis of microvilli was involved in the virus particles so as to form a virus core. MOORE recently revised his opinion and denied the transformation of unripe particles to superficial ripe ones[28)]. Two theoretical views ("one that virus particles are made of cell surface membrane and another that they are of intracytoplasmic origin") are now presented on the basis of knowledge of virus particles in malignant animal tumors. It is known that virus particles have unit membranes morphologically and in malignant tumors they range from 70 to 90 mμ in diameter. It is also said that nucleic acid in benign tumors is DNA, while in malignant tumors it is RNA[1)2)].

The aggregated particles observed by the author in the cytoplasm of both the synovial sarcoma and the malignant parotid tumor, sometimes extended into the extracellular area. The particles in the synovial sarcoma were 70-80 mμ in diameter, resembling viruses in malignant tumors, while those in the malignant parotid tumor were 25-30 mμ and clumped together to form larger particles, 70-80 mμ in diameter. The staining picture of the particles observed in the present study is worthy of note. They stained well with lead-hydroxide or uranyl-acetate and could be seen without any staining. Since uranyl-acetate stained them most clearly they are considered to be composed chiefly of nucleic acid, since other substances stain only very lightly with uranyl-acetate.

The aggregations of particles 70 to 80 mμ in width, detected by the author in the synovial sarcoma may be considered virus particles, because no such particles develop in normal cells. It may be too soon to draw final conclusions from these observations, but these particles must, at least, be closely related to viruses.

The virus-like particles in the malignant parotid tumor were each 20-30 mμ in diameter but they formed aggregates 70-80 mμ in diameter. These aggregates stained strongly with uranyl-acetate, though less strongly than the synovial sarcoma. Their staining indicated that they were composed mainly of substances other than nucleic acids. These nucleic acid particles are never observed in normal cells; i. e. normal cells contain no virus or virus-like particles Z. OTA has described virus-like particles within the cytoplasm, so viruses may be located within cells as well as on the cell mambrane. The present study leads the author, also, to conclude that, in some instances, viruses are present in the cytoplasm.

These findings suggest that viruses may cause non-epithelial malignant tumors in some cases, and that treatment with roentgenrays or anticarcinoma drugs may be necessary to demonstrate them. For example, in chicken sarcoma cells the causative virus could be seen only after irradiation with 200 γ.

The case of synovial sarcoma, in which virus-like particles were demonstrated by the author, had received 24 roentgen treatments (a total of 6,000 γ) and 14 injections of 0.5 mg of tespamin (N•N′•N″ Triethylen Thiophosphoramide), whereas the case of malignant mixed parotid tumor had received no specific therapy before surgery. In a patient with reticulosarcoma, who had received 13 roentgen treatments (totaling 3,800 γ) and nitromin (Methyl bis-(β-chloroethyl)-amine-N-oxied hydrochloride) (12 injections of 50 mg each) and in another patient with seminoma treated with nitromin (Methyl bis-(β-chloroethyl)-amine-N oxied hydrochloride) (8 arterial injections of 50 mg each), the author failed to demonstrate virus. These observations suggest that if the particles themselves are viruses, the strength of treatment may influence greatly the appearance of the virus.

CONCLUSION

Thirty five human tumors were studied with the electron microscope. They consisted of 22 non-epithelial malignant tumors, 4 epithelial malignant tumors, 1 malignant mixed tumor, 2 non-epithelial benign tumors, 1 benign mixed tumor, and 5 nodes with lymphadenitis. The author detected virus-like particles in a synovial sarcoma and in a malignant parotid tumor. The majority of particles were situated within the cytoplasm but a few were extracellular.

The particles in the synovial sarcoma were 70-80 mμ in diameter, resembling virus particles which had previously been identified in malignant tumors. Those in the malignant mixed parotid tumor were 25-30 mμ in diameter, but they clumped together to form larger aggregates. No unit membrane was seen in either case. The particles were stained strongly with uranyl-acetate, indicating that nucleic acid was the essential component. As such nucleic acid particles never appear in normal cells, the particles observed in these two cases may be considered to be virus particles or entities closely associated with viruses.

In order to demonstrate virus particles in tumor cells, treatment with roentgen-rays or anticarcinoma drugs may be necessary, and the strength of such treatment may influence the appearance of virus particles.

ACKNOWLEDGEMENT

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(1) Synovial sarcoma

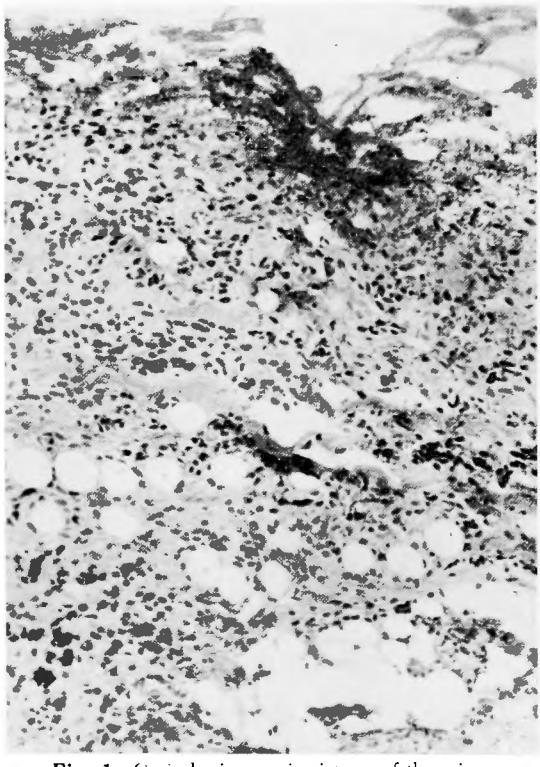


Fig. 1 Optical microscopic pictures of the primary tumor extirpated on January, 24th, 1962. Electronmicroscopic observation was not performed with it.

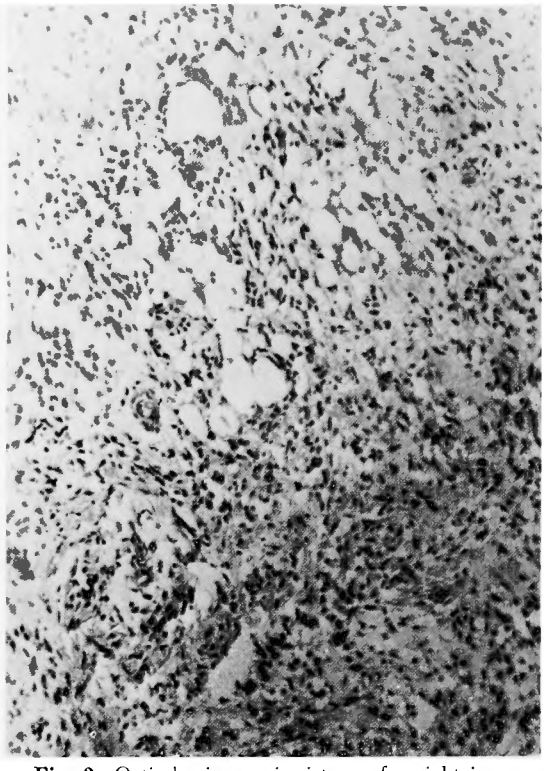


Fig. 2 Optical microscopic pictures of a right inguinal lymphnode metastasis extirpated and cleaned-up on June, 25th, 1962. Figs. 4–17 were electron micrographs of the metastasis.

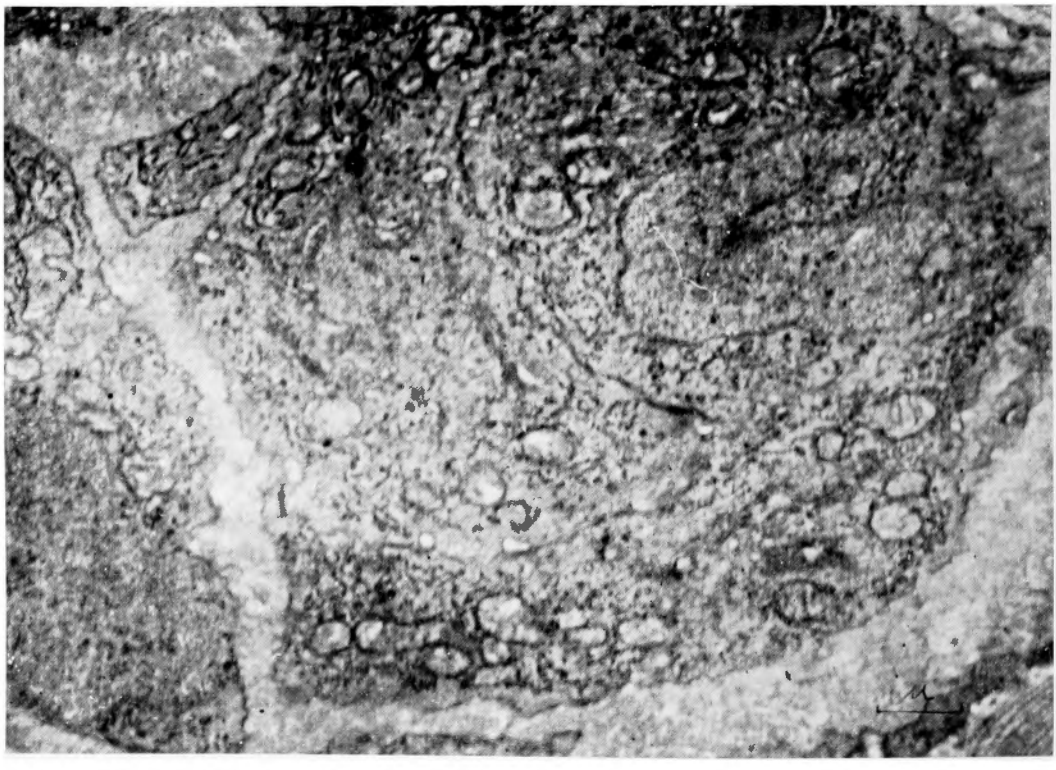


Fig. 3 Electron microscopic pictures (Non-staining) of lymphadenitis served as the control. Well-developed mitochondria and endoplasmic reticulum were observed, but particles were not observed.

Electron Microscopic Photogram of the Synovial Sarcoma (Figs. 4-17).

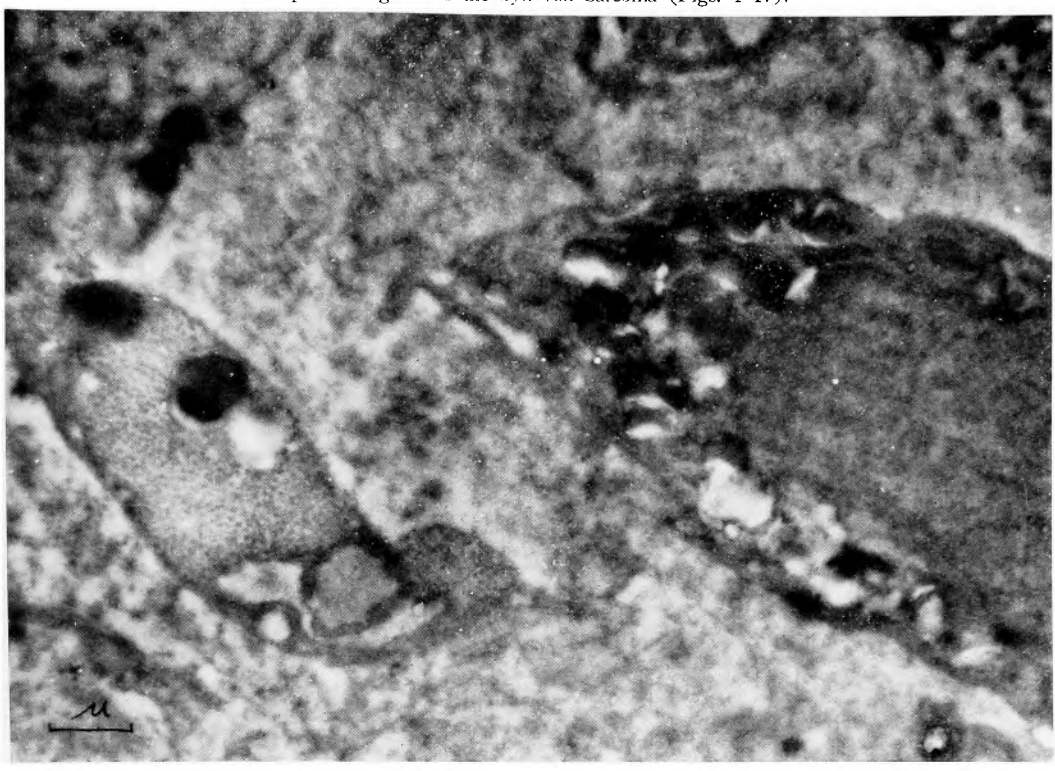


Fig. 4 Non-staining. Development of mitochondria and endoplasmic reticulum was markedly inhibited. Electron-dense particles filled up the cytoplasm. Vacuoles also were seen throughout it.

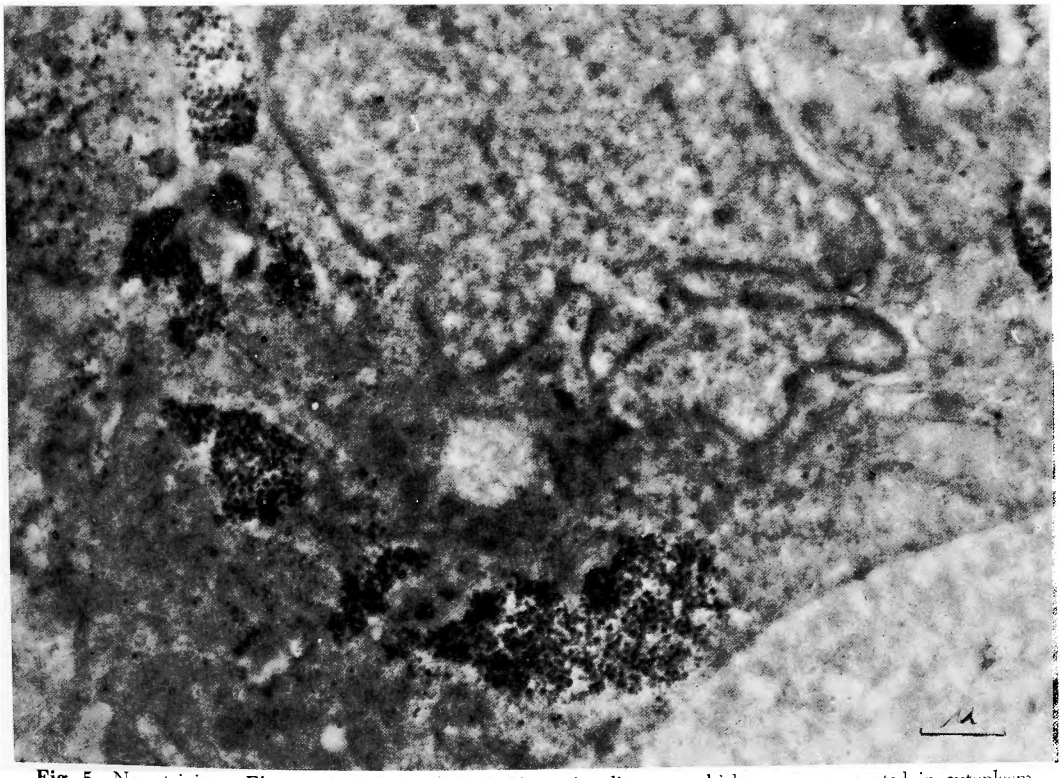


Fig. 5 Non-staining. Electron-dense particles, 70-80 mμ in diameter which were aggregated in cytoplasm. Mitochondria and endoplasmic reticulum developed deficiently.

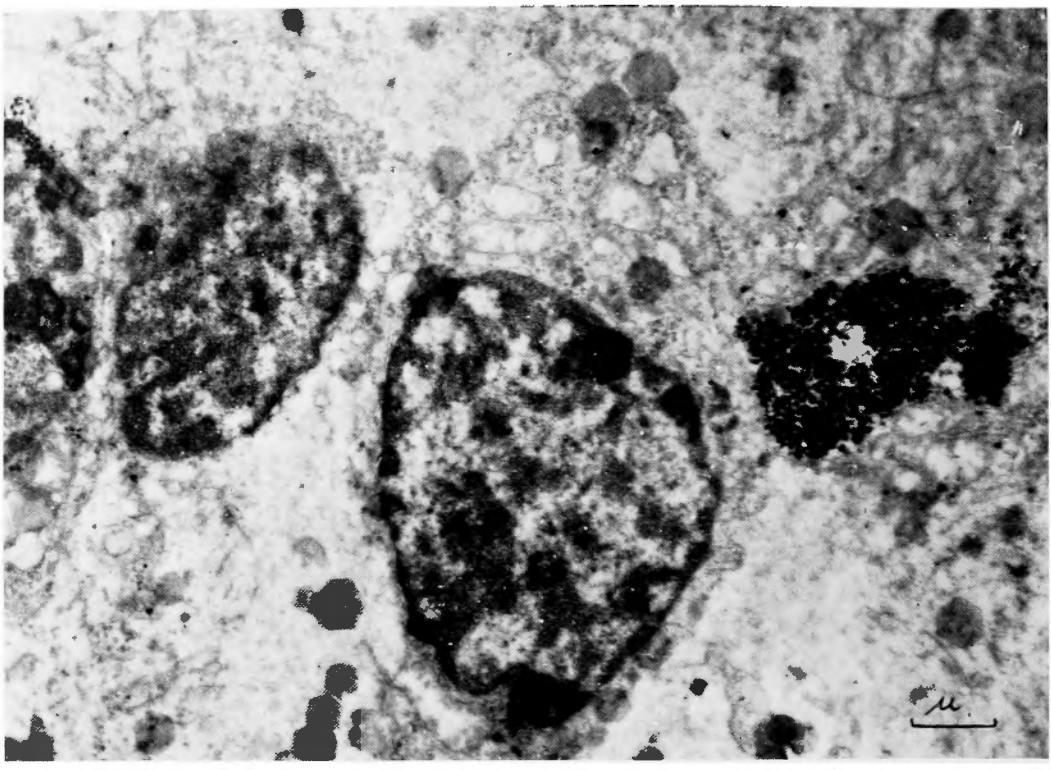


Fig. 6 Lead-hydroxide staining. Mitochondria developed were, but endoplasmic reticulum did poorly. Moderately stained particles were aggregated in cytoplasm.

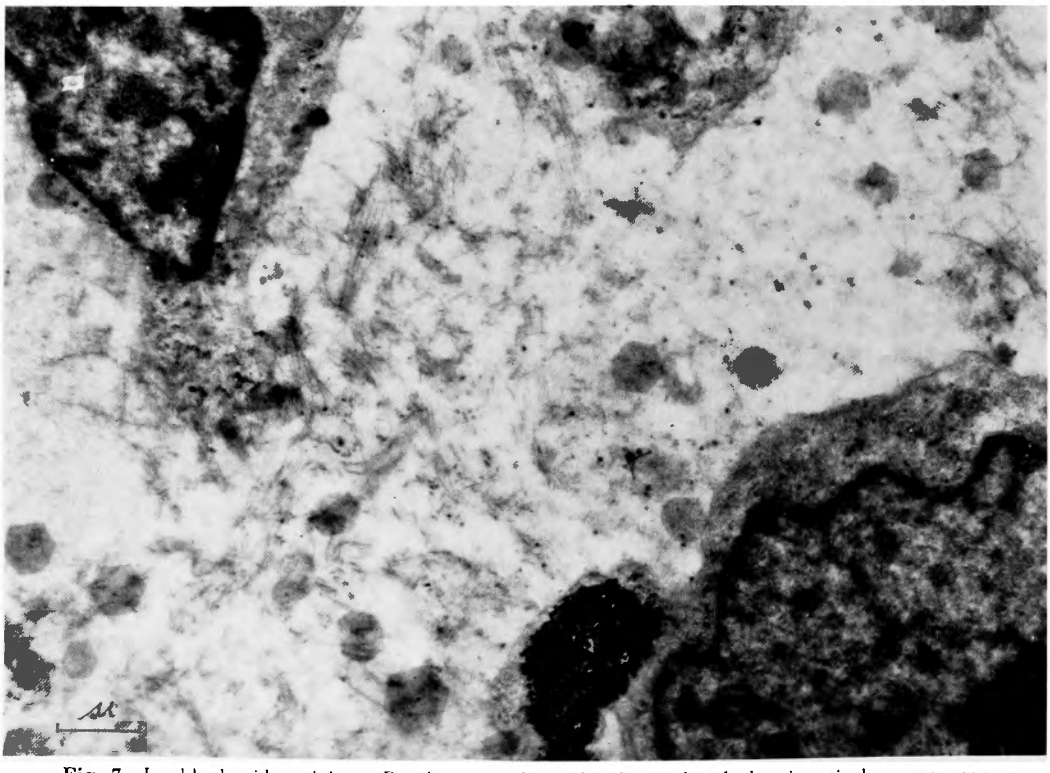


Fig. 7 Lead-hydroxide staining. Development of mitochondria and endoplasmic reticulum was poor. Moderately stained particles appeared in cytoplasmal clotting each other.

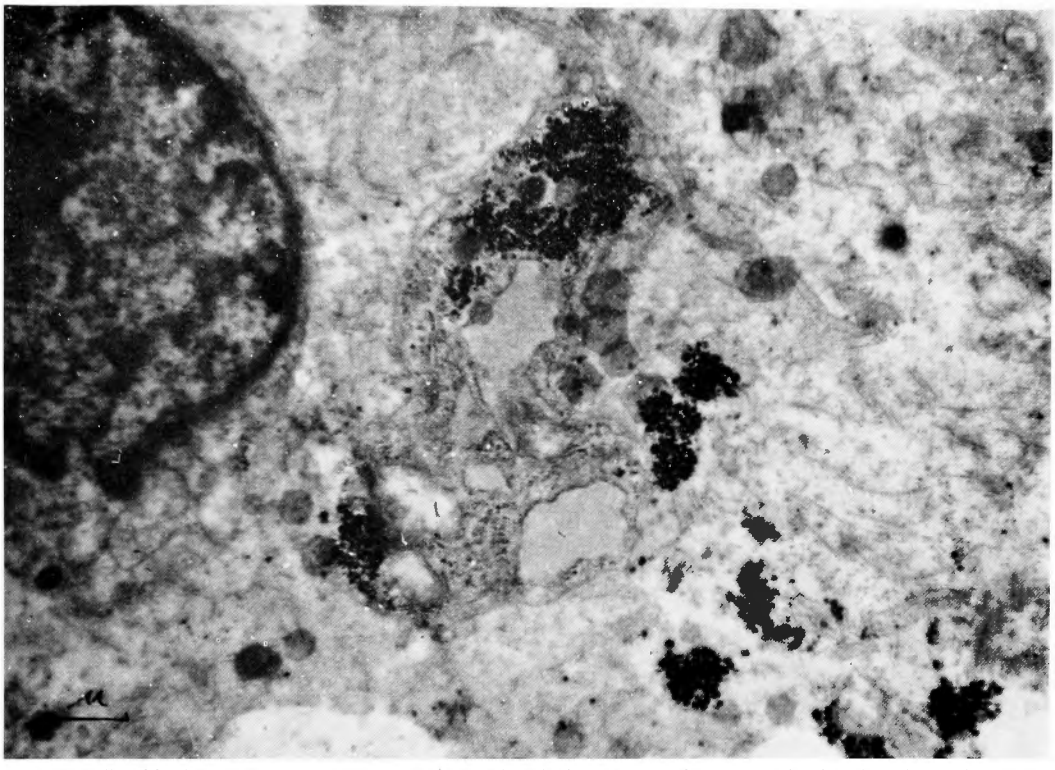


Fig. 8 Lead-hydroxide staining. Mitochondria and endoplasmic reticulum were developed rather deficiently, and vacuoles were produced in large number. Particles in group well-stained, were distributed chiefly in the cytoplasm and some of them appeared in the extracellular space.

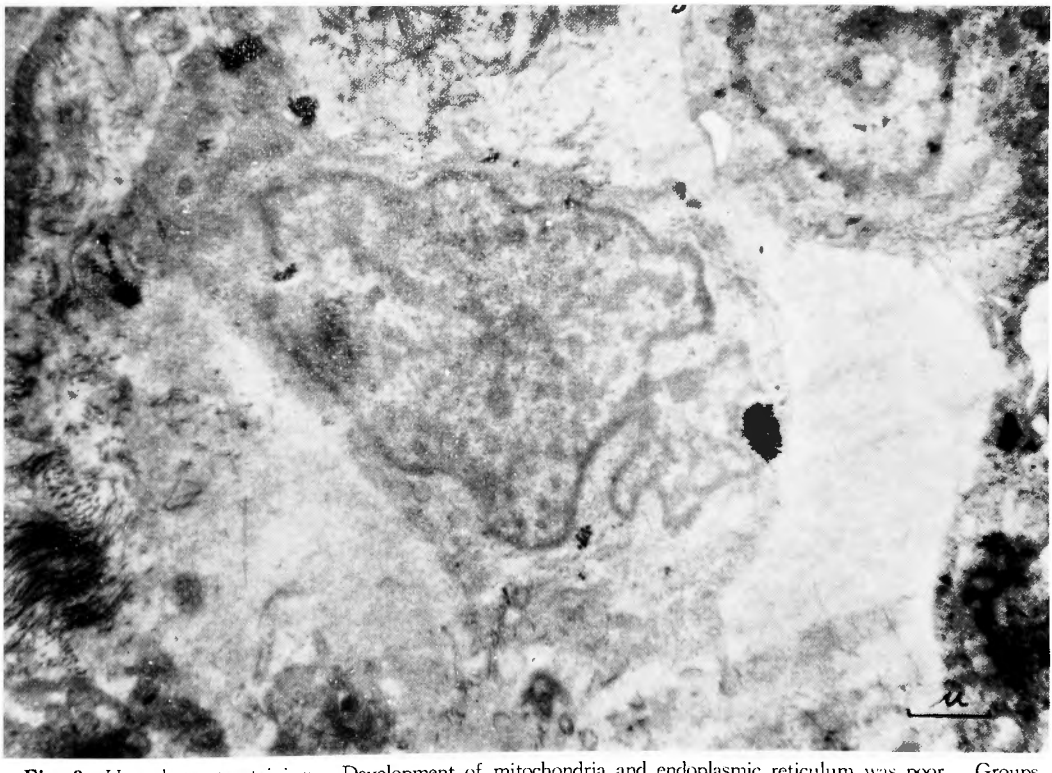


Fig. 9 Uranyl-acetate staining. Development of mitochondria and endoplasmic reticulum was poor. Groups of heavily stained particles were seen in the cytoplasm.

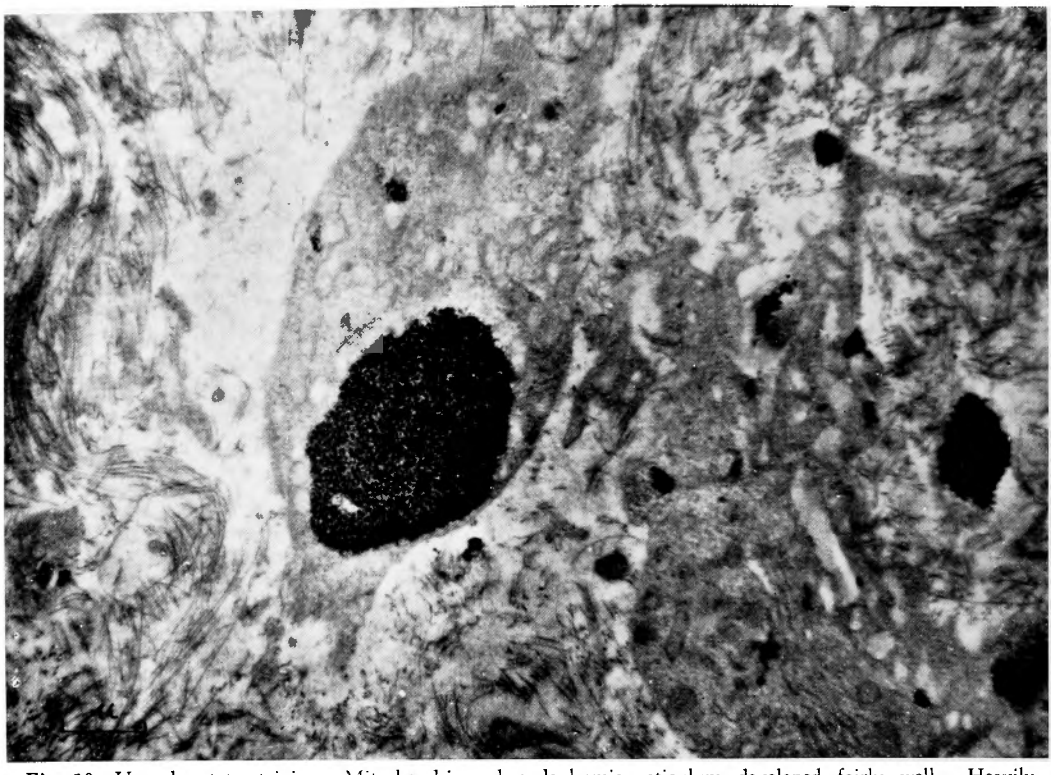


Fig. 10 Uranyl-acetate staining. Mitochondria and endoplasmic reticulum developed fairly well. Heavily stained particles aggregated in cytoplasm and some of them appeared in extracellular space.

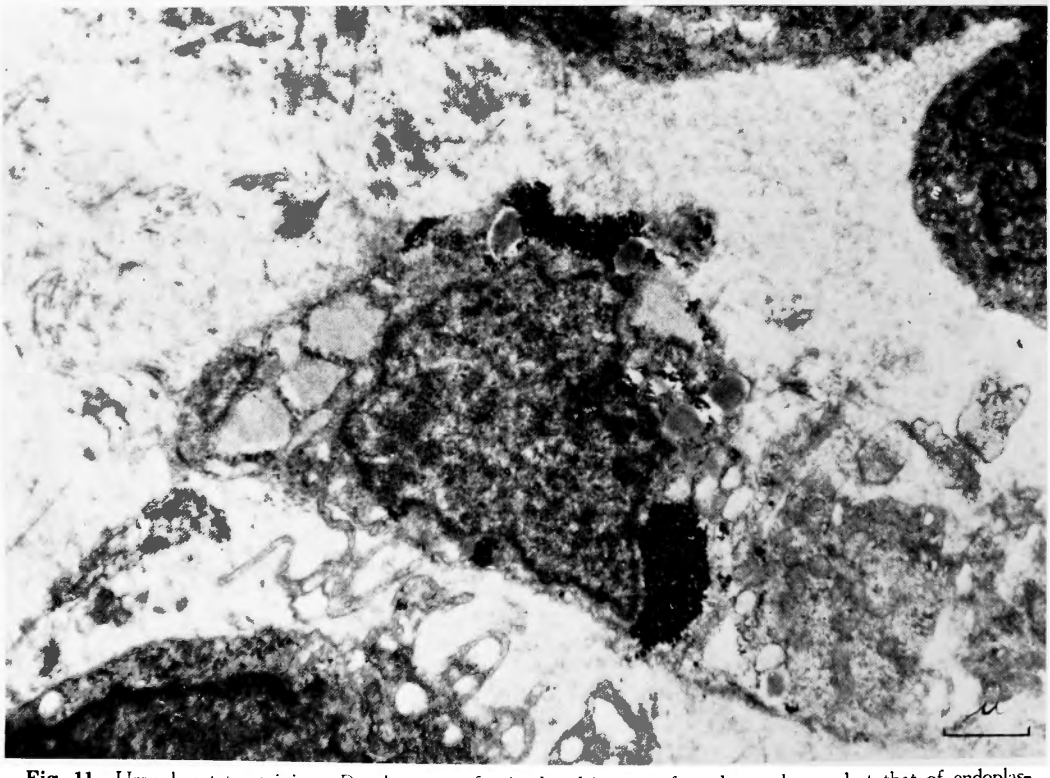


Fig. 11 Uranyl-acetate staining. Development of mitochondria was of moderate degree, but that of endoplasmic reticulum was poor. Numerous vacuoles were produced in the cytoplasm. Heavily stained particles crowded in the cytoplasm and some of them made such appearance as if they were getting out of the cell into extracellular space.

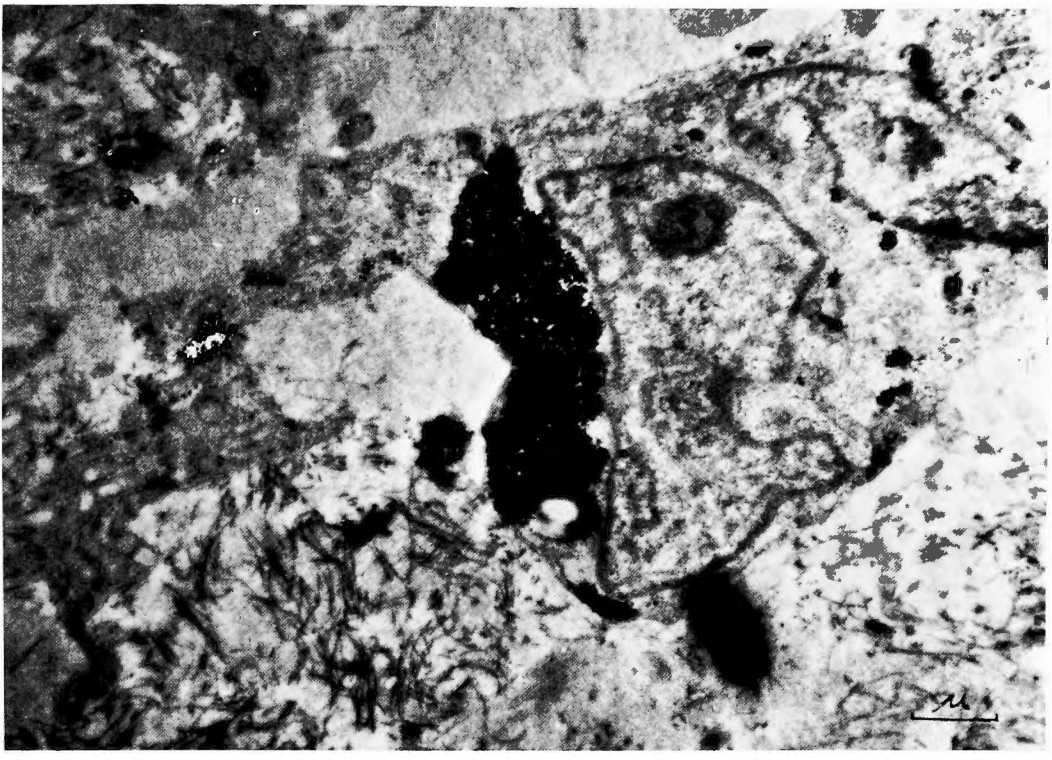


Fig. 12 Uranyl-acetate staining. Mitochondria and endoplasmic reticulum developed poorly with many vacuoles. Heavily staining particles, 70–80 mμ in size, were accumulated in the cytoplasm.

Fig. 13 Uranyl-acetate staining. Development of mitochondria was of moderate degree and that of endoplasmic reticulum was poor. Considerable number of vacuoles were seen. Aggregation of heavily stained particles were demonstrated in the cytoplasm.

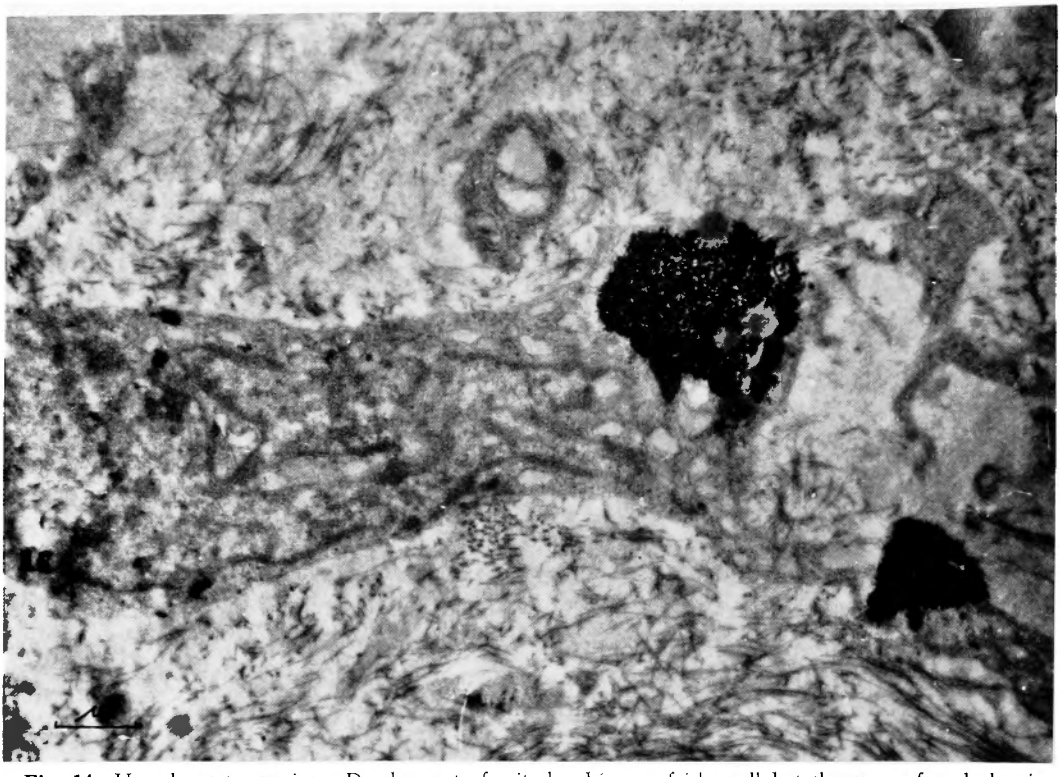
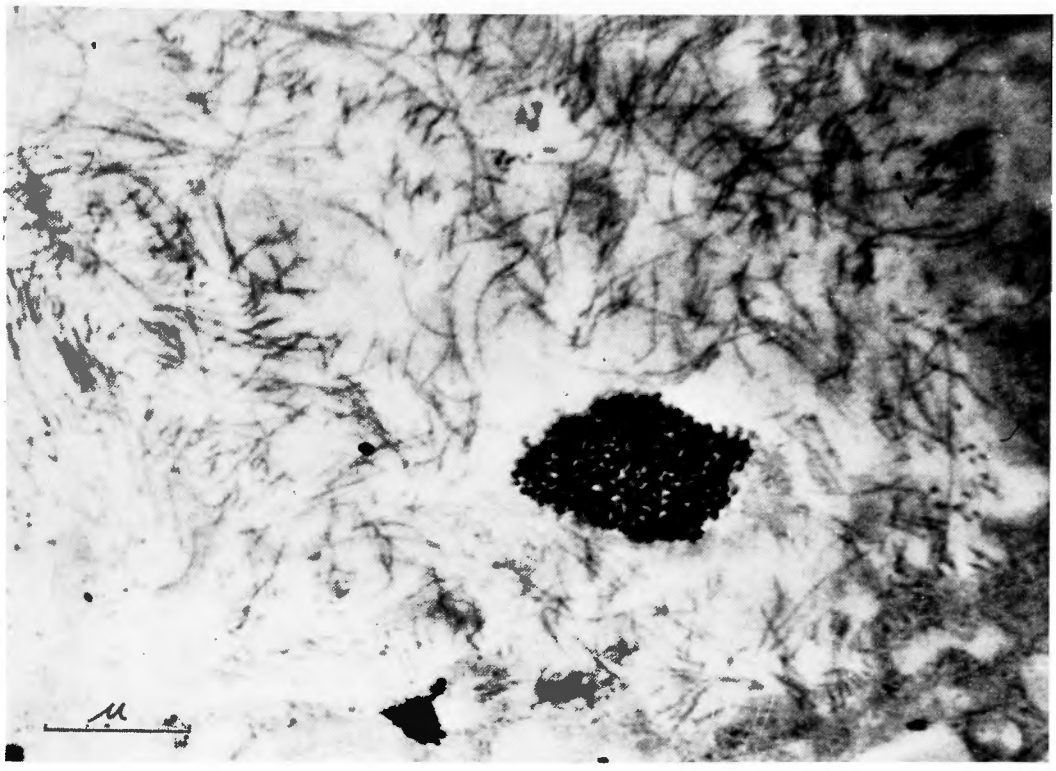


Fig. 14 Uranyl-acetate staining. Development of mitochondria was fairly well but the same of endoplasmic reticulum was strongly poor. Many vacuoles were produced. Aggregation of heavily stained particles were demonstrated in the cytoplasm.

Fig. 15 Uranyl-acetate staining. Rather poor growth of mitochondria, fairly developed endoplasmic reticlum and many vacuoles were seen. Heavily stained particles crowded together into cytoplasm so markedly that the nucleus was disfigurated by the squeeze of them. Particles also protruded from the cell into extracellular space.



Fis. 16 Uranyl-acetate staining. Heavily stained particles, 70–80 mμ in size, aggregated in extracellular space.

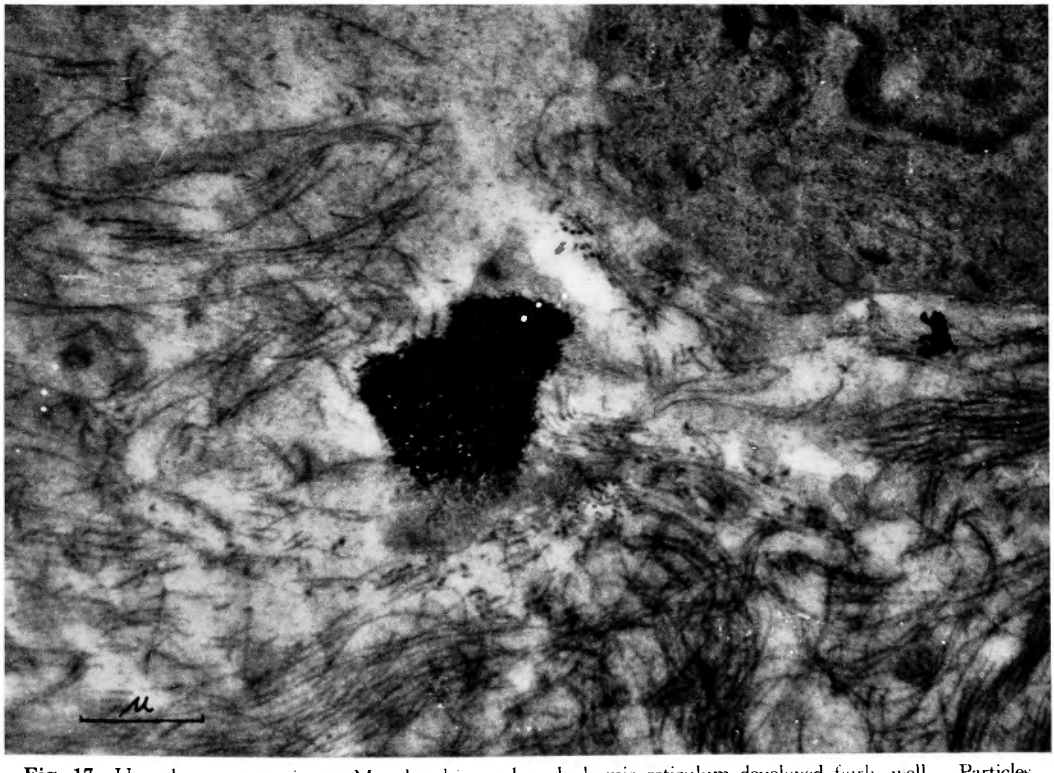


Fig. 17 Uranyl-acetate staining. Mitochondria and endoplasmic reticulum developed fairly well. Particles aggregated in the cytoplasm and majority of them sticked out on the cytoplasm into extracellular space.

(2) Malignant Mixed Parotid Tumor

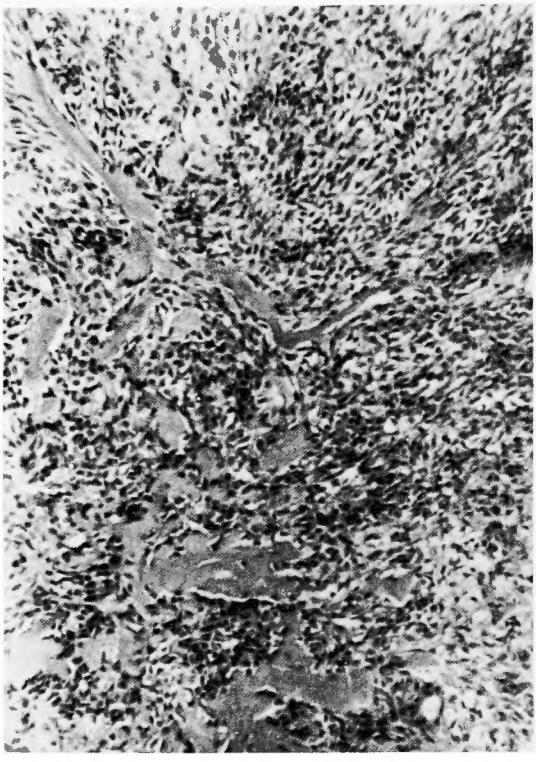


Fig. 18 Optical microscopic picture of the primary tumor developed in right infraauricular area, operated on Janurary, 24th, 1962. Figs. 21-29 concern electron microscopical photograms of the specimen.

Fig. 19 Benign mixed parotid tumor served as the control. Well-developed mitochondria, poorly developed endoplasmic reticulum and no particle were seen.

Figs. 20-26 Shows electronmicroscopical photograms of the malignant mixed parotid tumor.

Fig. 20 Non-staining. Mitochondria fully developed but endoplasmic reticulum did deficiently. Particles 25-30 mμ in width were seen in the cytoplasm.

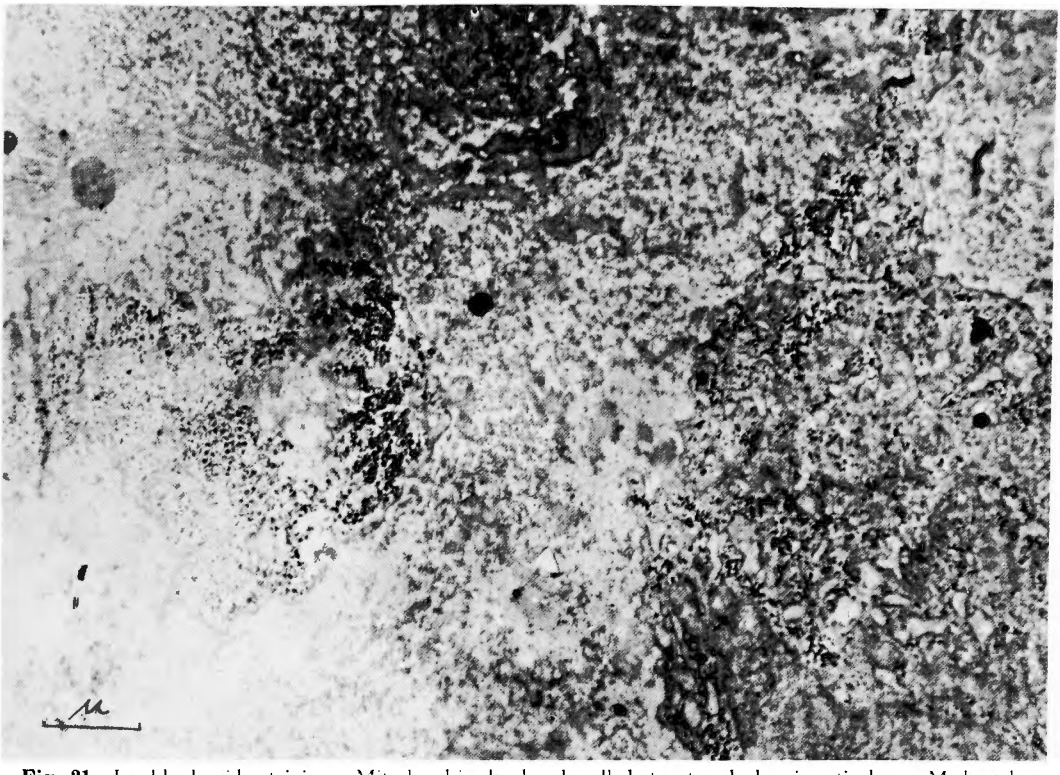


Fig. 21 Lead-hydroxide staining. Mitochondria developed well, but not endoplasmic reticulum. Moderately stained particles 20–30 mμ in diameter were seen.

Fig. 22 Lead-hydroxide staining. Mitochondria developed fairly well but endoplasmic reticulum did poorly. Particles stained with a considerable contrast were seen in the cytoplasm.

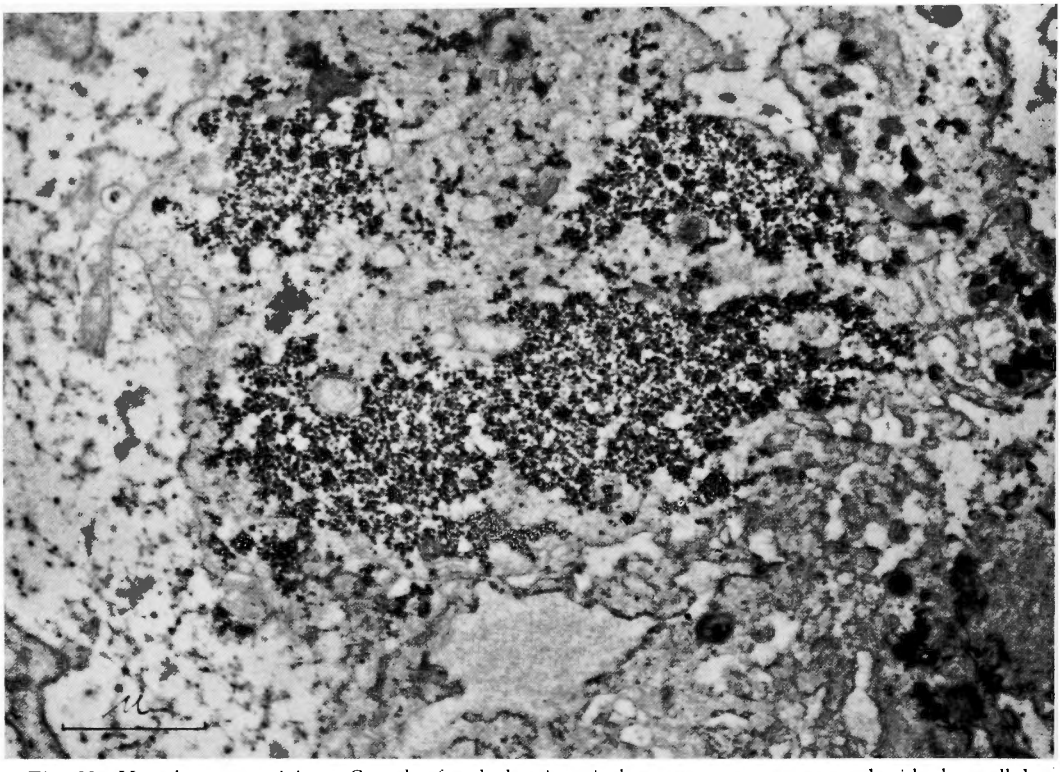


Fig. 23 Uranyl-acetate staining. Growth of endoplasmic reticulum was poor as compared with the well-developed mitochondria. Intensively stained particles were demonstrated in the cytoplasm.

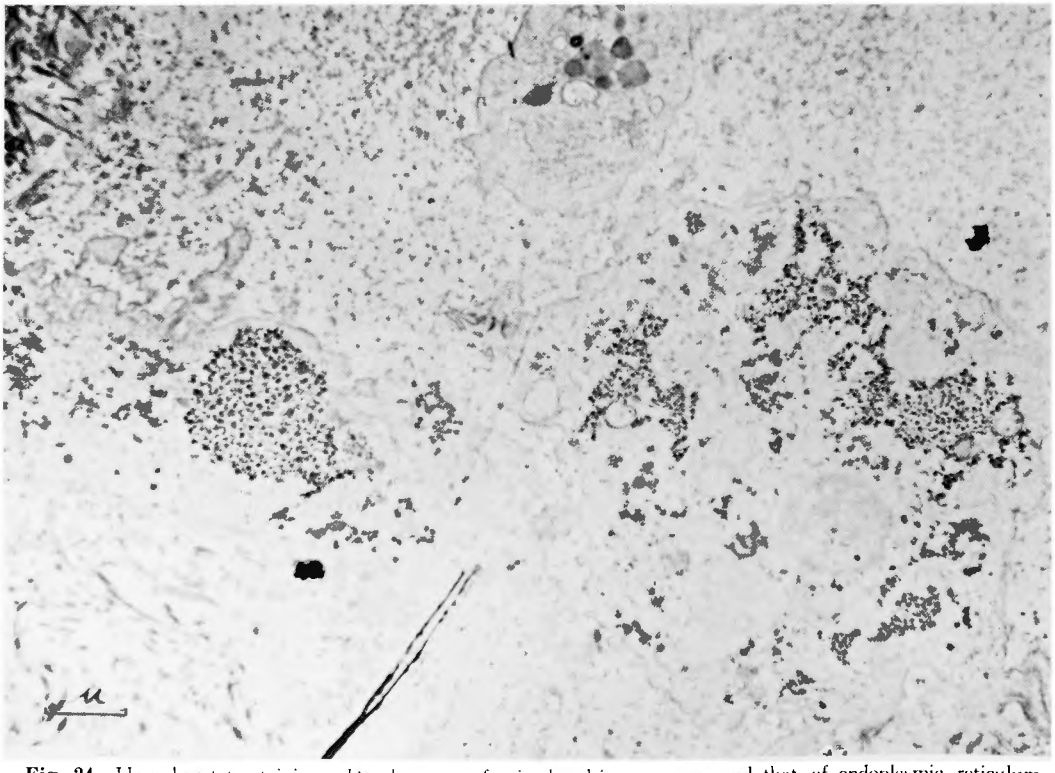


Fig. 24 Uranyl-acetate staining. Development of mitochondria was poor and that of endoplasmic reticulum was moderate. Particles stained with a considerable contrast were proved in the cytoplasm.

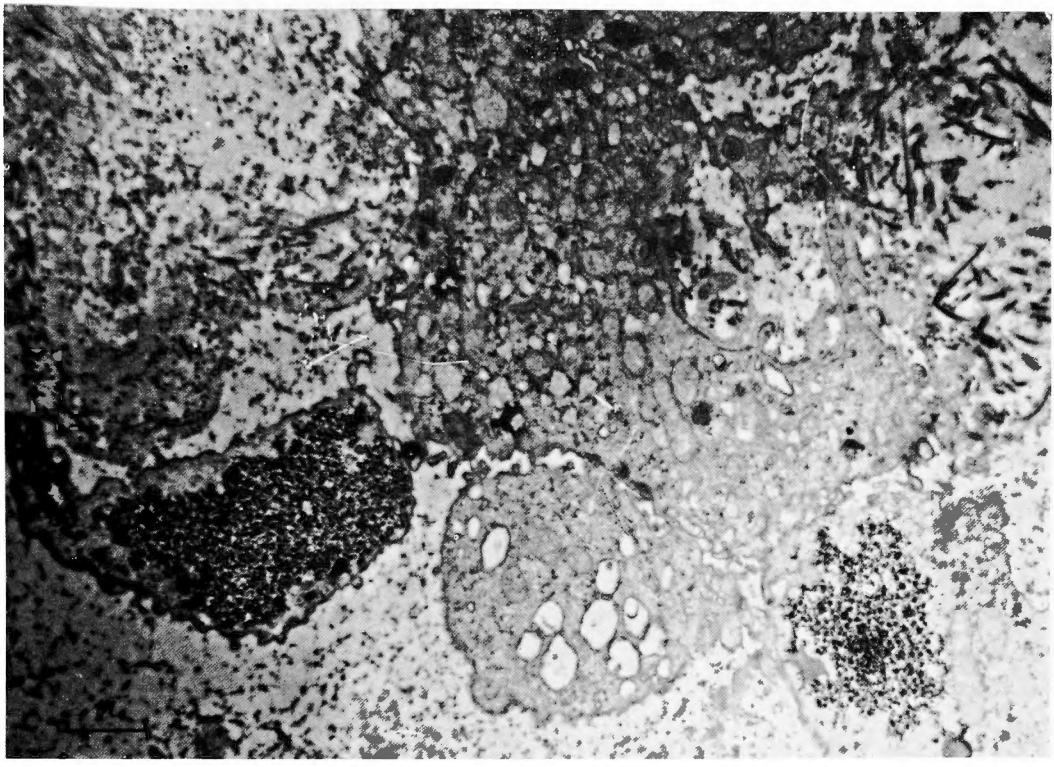


Fig. 25 Uranyl-acetate staining. Mitochondria developed markedly with many vacuoles, while endoplasmic reticulum developed poorly. A number of particles 25–30 mμ in width and considerably well-stained, were distributed in the cytoplasm. Some of these particles were clotted to form larger particles of 70–80 mμ in diameter.

Fig. 26 Uranyl-acetate staining. Mitochondria and endoplasmic reticulum developed moderately. Particles, stained to a considerable intensity, were seen in cytoplasm. These particles were 25–30 mμ in width but built up larger paeticles (70–80 mμ) due to their aggregation.

和文抄録

人間の非上皮性悪性腫瘍に於ける電子顕微鏡学的研究

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近時腫瘍ウィルスの研究がすすみ，種々のウィルス性腫瘍が動物で見出されてきた．又，人間に於ても，腫瘍ウィルスの発見の報告が2，3見られる．

私も人間の非上皮性悪性腫瘍22例，上皮性悪性腫瘍4例，悪性混合腫瘍1例，非上皮性良性腫瘍2例，良性混合腫瘍1例，淋巴腺炎5例について電子顕微鏡学的研究を行ない，その中滑液嚢肉腫の1例，及悪性混合性耳下腺腫瘍1例に，大部分は細胞質内に，一部細胞外にある小顆粒の群集を観察した．滑液嚢肉腫の小顆粒の大きさは70及至80 mμで，一般に云われる悪性腫瘍のウィルス顆粒の大きさとほぼ一致するが，悪性混合性耳下腺腫瘍の小顆粒の大きさは25及至30 mμで，これ等の小顆粒が集り，70及至80 mμの小顆粒を形成している．この二者に於ては，いづれも二重膜構造を認める事は出来なかつた．しかしこれ等を水酸化鉛，及ウラニールアセテートにて染色するに，水酸化鉛にもかなり染色されるが，ウラニールアセテートには非常に高度に染色される．この染色性から見て，これ等の小顆粒は核酸により形成されていると思われ，細胞質内にこの様な核酸顆粒の群集の存在は正常の細胞では考えられず，ウィルス顆粒と断定することはこれのみでは危険ではあるが，少くともウィルスと深い関係にあるものと考えられる．

腫瘍ウィルスを電子顕微鏡的に観察する場合，レントゲン線照射，又は制癌剤注射等何か誘導処理が必要ではないかと思われる．家鶏肉腫に於ても誘導処理を行なつていない標本ではほとんどウィルス顆粒は観察されず，200γのレントゲン照射が行なわれた時が一番よく観察される．私の研究に於ては，滑液嚢肉腫の患者は標本作製迄にレントゲン治療24回，計6,000γ．及テスパミン5 mg 14回注射を受けているが，一方悪性混合性耳下腺腫瘍の患者は全くこれらの治療を受けていない．又，細網肉腫の患者でレントゲン治療13回，計3,800γ，及ナイトロミン 50 mg12回注射を受けた者，及精虫腫の患者でナイトロミン50mg 8回動脈注射を受けた者等も電子顕微鏡で観察したが，いづれも小顆粒は認められなかつた．

これ等の事から誘導処理も量的関係が非常に強いものではないかと考えられる．